PetNAD™

Mycoplasma felis Detection Kit

User Manual

For Research Use Only

Manufacturer:

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INTENDED USE

PetNAD™ *Mycoplasma felis* Detection Kit is intended for *in vitro* detection of *Mycoplasma felis* (*M. felis*) based on insulated isothermal polymerase chain reaction (iiPCR) technology. This kit is designed specially to be used with an insulated isothermal (iiPCR)-compatible instrument, **POCKIT™** Nucleic Acid Analyzer. The assay is intended for use by veterinarians or technicians with basic laboratory skills.

This kit is intended for research use only.

SCIENTIFIC MEANINGS

Antibody induced by vaccine or obtained from maternal immunity could lead to false positive interpretation in antibody-based diagnostic procedures. Detecting pathogen's nucleic acids, not antibody, PCR-based methods can avoid the false positive results described above.

Furthermore, with higher analytical sensitivity, PCR can detect lower levels of viral signals than most if not all diagnostic methods. It can reduce the chance of false negative results at early infection stage and shorten the window period between time of infection and detection.

SUMMARY AND EXPLANATION

Mycoplasma spp. are gram-negative bacteria. Several *Mycoplasma* species are part of the normal bacterial flora of animals; many of them are regarded as harmless to animals. However, *M. felis* can be pathogenic to both cats and horses. In case of shelter cats, isolation of *M. felis* from oropharyngeal swabs has been associated with upper respiratory tract infection and conjunctivitis (Bannasch et al., 2005).

PCR is one of the most commonly accepted methods that provide high sensitivity and specificity for *M. felis* detection. However, conventional PCR assays could take three to four hours and require sophisticated thermocyclers and well-trained technicians to perform. GeneReach has developed **PetNAD™** *Mycoplasma felis* Detection Kit based on iiPCR technology, which significantly reduces reaction time and offers sensitivity and specificity comparable to those of conventional nested PCR (Tsai, 2012; Chang, 2012). Furthermore, this simple and easy assay is completed rapidly in a portable **POCKIT™** Nucleic Acid Analyzer.

PRINCIPLES OF THE PROCEDURE

In iiPCR, hydrolysis probe-based chemistry is used to generate fluorescent signal during amplification of target gene. The primers and probe target the *gag* gene and do not cross-react with nucleic acid from host and other feline pathogens.

PRODUCT DESCRIPTION

A. Materials Provided (24 tests/kit)

Component	Contents or Purpose	Amount
Premix Pack	■ *M. felis* Premix (lyophilized pellet) containing dNTPs, primers, probe, and enzyme for amplification. ■ Desiccating agent pack.	24 bags (1 *M. felis* Premix vial and 1 desiccating agent/bag)
Premix Buffer B	■ Reaction buffer to re-dissolve the lyophilized pellet.	2 vials (1.3 ml/vial)
P(+) Control	■ Dried plasmid containing *M. felis* partial sequence as positive control.	1 vial
P(+) Control Buffer	■ Reaction buffer to re-dissolve P(+) Control.	1 vial (110 µl/vial)
R-tube		1 bag (24 pieces/bag)

Cap		1 bag (24 pieces/bag)
User Manual		1 copy

B. Materials and Equipment Required, but Not Provided

1) **PetNAD™** Nucleic Acid Co-prep Kit or **taco™** Automatic Nucleic Acid Extraction System.
2) **POCKIT™** Nucleic Acid Analyzer (**POCKIT™**): **PetNAD™-**compatible instrument.
3) **cubee™** Mini-Centrifuge (**cubee™**).
4) Micropipette and filter tips.

C. Storage and Stability

1) The kit should be stored at 4℃ and is stable until the expiration date stated on the label.
2) Store Premix vials in sealed Premix Pack to avoid hydration of lyophilized components.
3) Reconstituted P(+) Control is stable for 6 months at 4℃. Aliquot reconstituted P(+) Control to avoid degradation of nucleic acid.

D. Sample Type

Nucleic acid extracted from ocular swab or oropharyngeal swab.

RECOMMENDED NUCLEIC ACID EXTRACTION METHODS

A. **PetNAD™** Nucleic Acid Co-prep Kit.

B. **taco™** DNA/RNA Extraction Kit, compatible instrument—**taco™** Automatic Nucleic Acid Extraction System.

Note: Please follow the instruction manual of above extraction methods to obtain optimal results. It is the user's responsibility to validate the combination of this reagent set with nucleic acids extracted by other methods for any particular application.

PRECAUTIONS

A. Do not open R-tube(s) after reaction to prevent any carryover contamination.

B. Perform extraction and amplification in two independent spaces to minimize contamination.

C. Do not reuse R-tube and Premix.

D. Include the P(+) Control to:

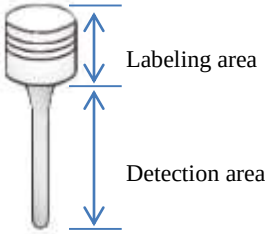
   1) Ensure **POCKIT™** is working normally.

   2) Ensure detection kit performance after storage.

E. To get optimal fluorescence detection.

1) Wear powder-free gloves to handle R-tubes.

2) Do not label in the detection area of R-tube.



LIMITATIONS

A. The test should be used only for testing nucleic acid extracted from animal specimens. Do not add specimens (*e.g.* whole blood) directly into Premix.

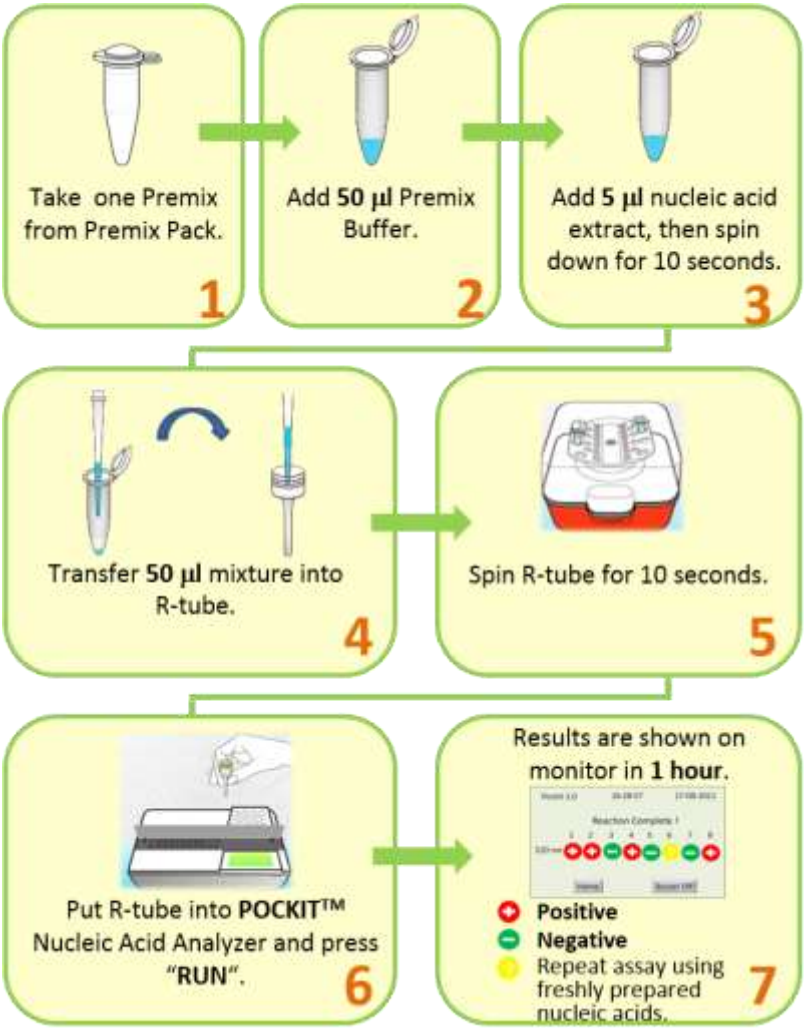
B. **PetNAD™** Nucleic Acid Co-prep Kit and **taco™ mini** Automatic Nucleic Acid Extraction System are recommended for nucleic acid extraction.

C. Any deviations from the recommended procedure may lead to suboptimal results. Quality of the extracts should be validated by the users.

D. For **PetNAD™** *Mycoplasma felis* Detection Kit, it is strongly recommended to use freshly prepared nucleic acid (within 1 hour after extraction) to achieve optimal results.

PROCEDURE

A. PetNAD™ *Mycoplasma felis* Detection Kit Quick Guide


Take one Premix from Premix Pack.
1
Add 50 µl Premix Buffer.
2
Add 5 µl nucleic acid extract, then spin down for 10 seconds.
3
Transfer 50 µl mixture into R-tube.
4
Spin R-tube for 10 seconds.
5
Put R-tube into POCKIT™ Nucleic Acid Analyzer and press "RUN".
6
Results are shown on monitor in 1 hour.
Positive
Negative
Repeat assay using freshly prepared nucleic acids.
7

B. Procedure

Note: Before preparing the reactions for iiPCR testing, turn on POCKIT™ to initiate the calibration for the instrument. The device will complete self-test within 5 minutes. Please refer to the user manual of POCKIT™ for further details.

Note: Before using for the first time, add 100 µl P(+) Control Buffer to P(+) Control. Store reconstituted P(+) Control at 4°C.

1) Label R-tube(s) in the label area.
2) Prepare one Premix for each sample. (Premix tube is in Premix Pack. Each Premix Pack contains one Premix tube.)

Note: When the pellet is not found at the bottom of the tube, spin tube briefly to bring it down.

3) Add 50 µl Premix Buffer B to each Premix tube.
4) Add 5 µl nucleic acid extract or P(+) Control to each Premix tube. Spin Premix tube for 10 seconds in a mini centrifuge (such as **cubee™**).
5) Transfer 50 µl Premix/sample mixture into R-tube.
6) Seal top of each R-tube with a cap. Make sure R-tube is capped tightly.
7) Place R-tube into the holder of **POCKIT™**.
8) Spin tube briefly in **cubee™** to make sure all solution is

collected at the bottom of R-tube.

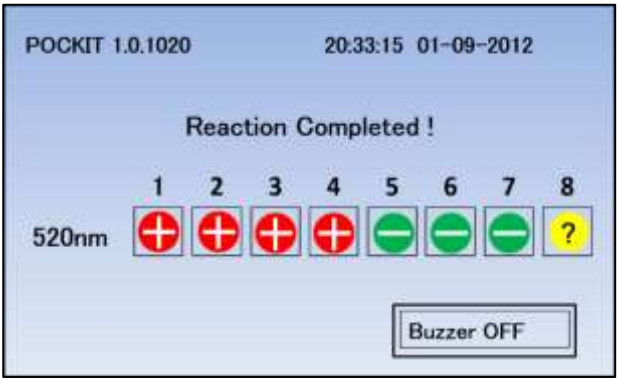
Note: Make sure there are no bubbles in the solution.

Note: Start reaction within 1 hour (to prevent nucleic acid degradation and non-specific reaction).

9) **POCKIT™** reaction:
   a) Select ″520 nm″.
   b) When ″System READY″ is displayed, place the holder with R-tube(s) into the reaction chamber.
   c) Tap cap of each R-tube to make sure the tube is positioned properly.
10) Close lid and press ″Run″ to start reaction program.
11) Test results are shown on the monitor after reaction is completed.

DATA INTERPRETATION

* One example of results shown on the monitor.



520 nm	Interpretation
⊕	*M. felis* Positive.
⊖	*M. felis* Negative.
?	Repeat reaction with freshly prepared nucleic acid.

ANYLYTICAL SENSITIVITY

The detection limit of **PetNAD™** *Mycoplasma felis* Detection Kit is about 10 copies/reaction.

TROUBLESHOOTING

Problems	**Possible causes**	**Solutions**
False Positive	1) Reuse of micro-centrifuge tubes, tips, R-tubes and Premix.	■ Micro-centrifuge tubes, tips, R-tubes and Premix are for single-use only. Reusing these accessories would cause cross-contamination, and therefore false positive results. ■ Used micro-centrifuge tubes, tips, R-tubes and Premix should be collected and discarded according to local regulation. Do not place the waste close to the working area to prevent cross-contamination.
	2) Contaminated micropipette	■ Use aerosol-free tips.
	3) Contaminated reagent	■ Consult with a GeneReach technical support representative or local distributor.
	4) Contaminated working area	■ Consult with a GeneReach technical support representative on how to clean up working area.

Problems	**Possible causes**	**Solutions**
False Negative	1) Nucleic acid extraction failed.	■ Consult manual of nucleic acid extraction kit.
	2) PCR inhibition	■ Do not overload PCR with too much nucleic acid. ■ Spike nucleic acid sample (5 µl) into a P(+) Control reaction for a parallel PCR reaction. Negative results indicate the presence of inhibitors in the nucleic acid. In that case, prepare another nucleic acid extract.
Heavy contamination of amplicons in reaction chamber of **POCKIT™**.	1) Leakage or spill of reaction from R-tube into reaction chamber of **POCKIT™**.	■ Consult with a GeneReach technical support representative or local distributor.

REFERENCE

1. Bannasch M.J., Foley J.E., (2005). Epidemiologic evaluation of multiple respiratory pathogens in cats in animal shelters. *J Feline Med Surg* 7:109–119.
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4. Tsai Y.L., Wang H.T.T., Chang H.F.G., Tsai C.F., Lin C.K., Teng P.H., Su C. and Jeng C.C., (2012) Development of TaqMan probe-based insulated isothermal PCR (iiPCR) for sensitive and specific on-site pathogen detection. *PLoS ONE* 7(9): e45278. doi: 10.1371/ journal. pone. 0045278